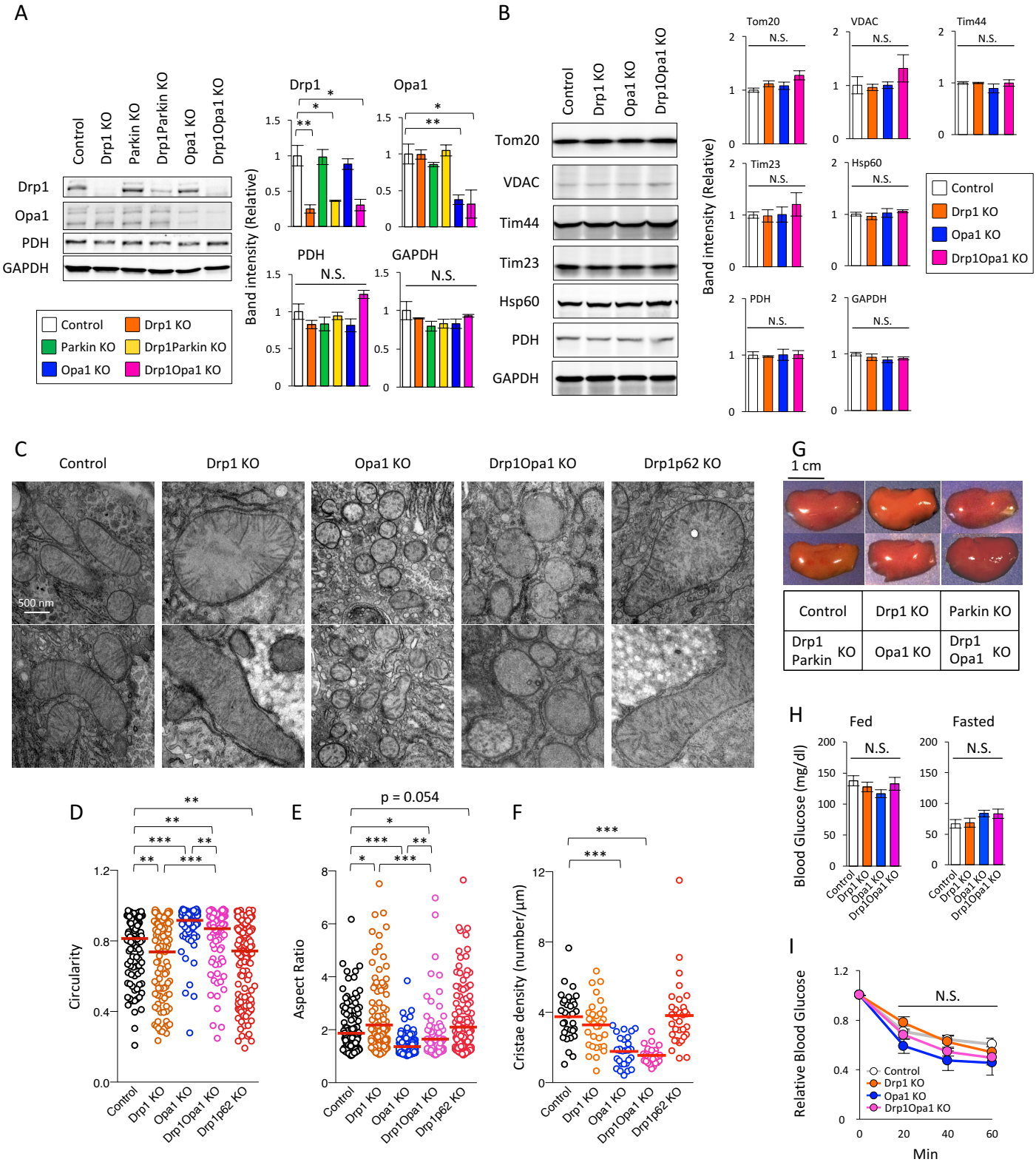
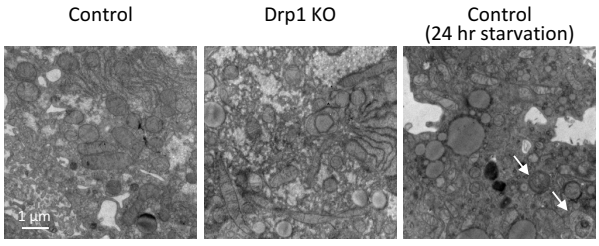

A
Control
Drp1 KO
Parkin KO
Drp1Parkin KO
Opa1 KO
Drp1Opa1 KO
Drp1
Opa1
PDH
GAPDH
Band intensity (Relative)
N.S.
B
Tom20
VDAC
Tim44
Tim23
Hsp60
C
Control
Drp1 KO
Opa1 KO
Drp1Opa1 KO
Drp1p62 KO
500 nm
D
Circularity
E
Aspect Ratio
p = 0.054
F
Cristae density (number/μm)
G
1 cm
Drp1 Parkin KO
Opa1 KO
Drp1 Opa1 KO
H
Fed
Fasted
Blood Glucose (mg/dl)
I
Relative Blood Glucose
Min

**Figure S1. Analysis of Alb-Drp1KO, Alb-Opa1KO and Alb-Drp1Opa1KO mice (Related to Figure 1).** (A and B) Western blotting of livers isolated from the indicated mice at 3 months of age using the indicated antibodies. Quantification of band intensity is shown. Values are average ± SEM (n=3 mice). (C) Mitochondria of the livers of the indicated mice at 3 months of age were examined by electron microscopy. (D) Circularity (n=128–167 mitochondria). (E) Aspect ratio (n=128–168 mitochondria). (F) The density of cristae along the inner membrane (n=26–32 mitochondria). (G) The general appearance of the liver is indistinguishable in control, Alb-Drp1KO, Alb-Opa1KO, Alb-Drp1Opa1KO, ParkinKO and Alb-Drp1ParkinKO mice. The left lobe is shown. (H) Blood glucose levels in fed and fasted mice (n=4–7 mice). (I) Insulin tolerance tests (n=6–8 mice). Glucose levels are normalized to those before insulin injection. Statistical analysis was performed using Student's *t*-test: *p< 0.05, **p< 0.01, ***p< 0.001.




| Number of autophagosome / 100 μm$^2$ | | |
|---|---|---|
| Control | Drp1 KO | Control (24 hr starvation) |
| 1.8±1.3 | 1.6±0.7 | 6.0±1.5 |

**Figure S2. Macroautophagy was not induced in Drp1KO livers (Related to Figure 2).** The livers of control and Alb-Drp1KO mice were analyzed by electron microscopy. As a positive control, macroautophagy was induced by starving control mice for 16 hs. Quantification of macroautophagosomes is shown (n=8–9 electron micrographs).

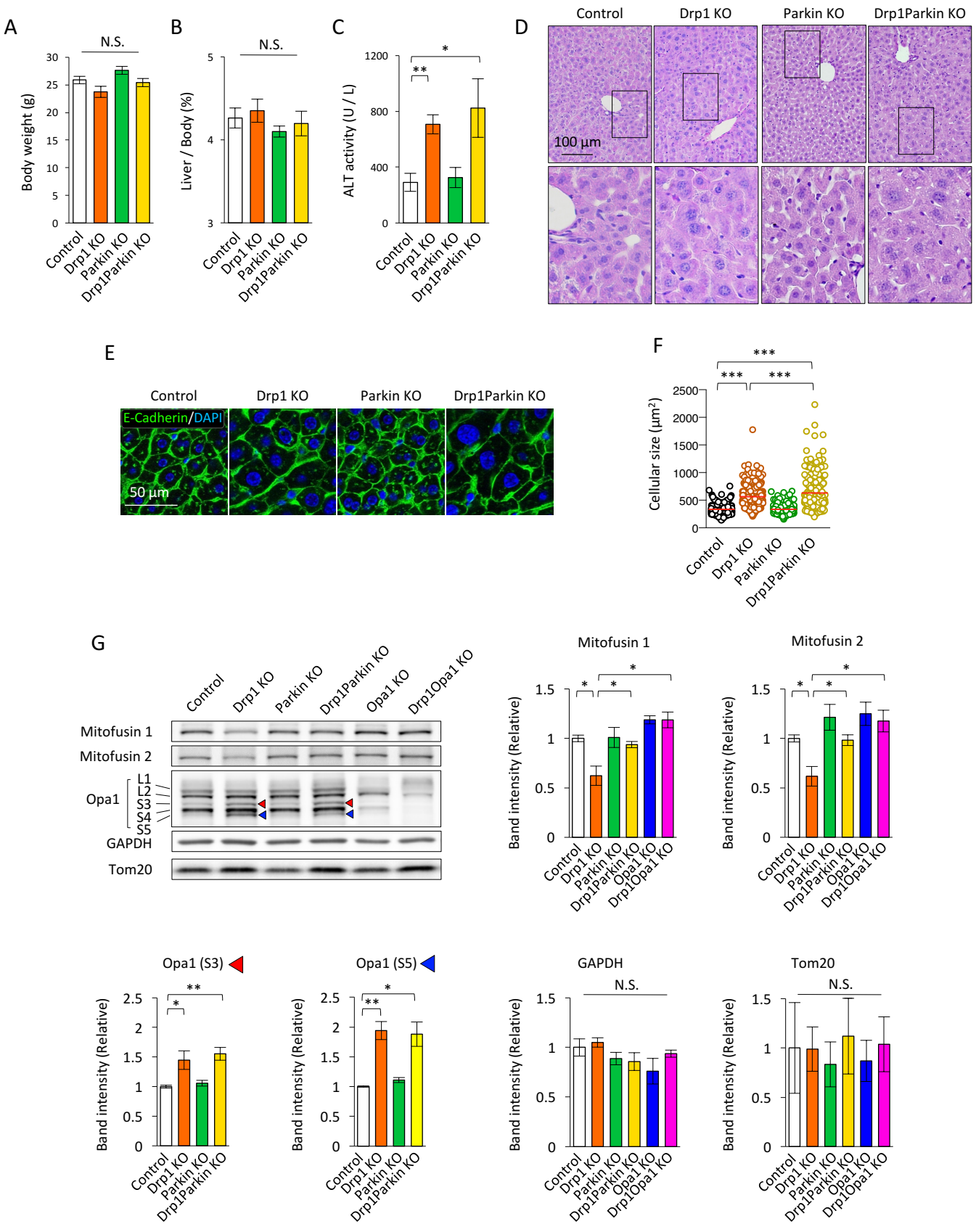
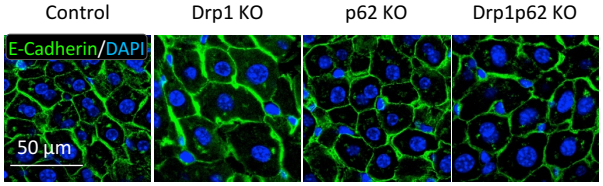
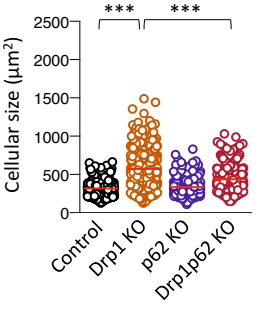

A
N.S.
Body weight (g)
30
25
20
15
10
5
0
Control
Drp1 KO
Parkin KO
Drp1Parkin KO
B
N.S.
Liver / Body (%)
5
4
3
C
*
**
ALT activity (U / L)
1200
800
400
0
D
Control
Drp1 KO
Parkin KO
Drp1Parkin KO
100 μm
E
Control
Drp1 KO
Parkin KO
Drp1Parkin KO
E-Cadherin/DAPI
50 μm
F
***
***
***
Cellular size (μm²)
2500
2000
1500
1000
500
0
G
Control
Drp1 KO
Parkin KO
Drp1Parkin KO
Opa1 KO
Drp1Opa1 KO
Mitofusin 1
Mitofusin 2
Opa1
L1
L2
S3
S4
S5
GAPDH
Tom20
Mitofusin 1
Mitofusin 2
Band intensity (Relative)
Opa1 (S3)
Opa1 (S5)
GAPDH
Tom20
N.S.

**Figure S3. The loss of parkin does not alleviate liver damage caused by Drp1 deficiency (Related to Figure 3).** (A) Body weights of the male mice. For the control and Alb-Drp1KO mice, the same data used in Fig. 1G are shown. Values are average ± SEM (n=3–6 mice). (B) Liver weight relative to body weight (n=5–7 mice). For the control and Alb-Drp1KO mice, the same data used in Fig. 1H are presented. (C) ALT levels in the blood. (n=4–5 mice). (D) PAS staining of liver sections. Boxed regions are enlarged. (E) Liver sections of the indicated mice were analyzed by confocal microscopy with anti-E-cadherin antibodies and DAPI. (F) Quantification of the size of cells. Red lines indicate averages (n=352 cells from 4 control mice, 264 from 3 Alb-Drp1KO mice, 264 from 3 ParkinKO mice, 264 from 3 Alb-Drp1ParkinKO mice). (G) Western blotting of livers isolated from the indicated mice at 3 months using antibodies to mitofusin 1 and 2, Opa1, GAPDH and Tom20. Quantification of band intensity is shown. Values are average ± SEM (n=3 mice). Statistical analysis was performed using Student's *t*-test: *p< 0.05, **p< 0.01, ***p<0.001.

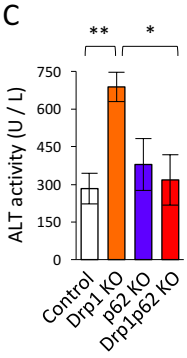
A




B




C

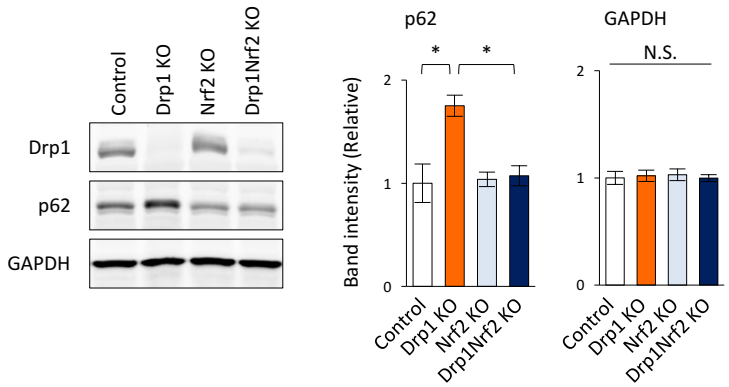



D

Relative mRNA expression of Nrf2 target genes

| | Nqo1 | Gsta1 | Gsta2 | Gsta3 | Gstm1 | Gclr | Gclc | Prdx1 |
|---|---|---|---|---|---|---|---|---|
| Drp1 KO | 0.8±0.2 | 3.1±1.2 | 0.9±0.3 | 0.7±0.2 | 1.6±1.0 | 1.1±0.4 | 1.7±0.6 | 1.3±0.6 |
| p value | 0.4 | 0.2 | 0.6 | 0.2 | 0.6 | 0.7 | 0.3 | 0.6 |

E




F

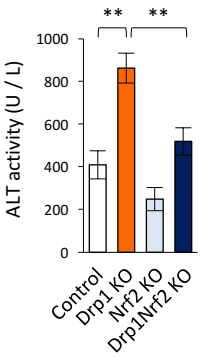


**Figure S4. The loss or downregulation of p62 mitigates liver damage caused by Drp1 deficiency (Related to Figure 6).** (A) Liver sections of the indicated mice were analyzed by confocal microscopy with anti-E-cadherin antibodies and DAPI. (B) Individual size of cells. Red lines indicate averages (n=294 cells from 3 control mice, 392 from 4 Alb-Drp1KO mice, 294 from 3 p62KO mice, 294 from 3 Alb-Drp1p62KO mice). (C) Blood levels of ALT. Values are average ± SEM (n=5 mice). (D) qRT-PCR analysis of Nrf2 target genes. Levels of mRNAs in Drp1KO livers were quantified relative to those in control livers. Albumin mRNA was used as an internal standard. Values are average ± SEM (n=3 mice). (E) Western blotting of livers isolated from control, Alb-Drp1KO, Nrf2-KO and Alb-Drp1Nrf2KO mice at 3 months of age using the indicated antibodies. Quantification of band intensity is shown. Values are average ± SEM (n=3 mice). (F) Blood levels of ALT. Values are average ± SEM (n=5 mice). Statistical analysis was performed using Student's *t*-test: *p< 0.05, **p< 0.01, ***p< 0.001.